to being in allowable condition, subject to two minor matters which are addressed below.

In the Office Action, the Examiner noted that the term "transgenic bovine species" is unclear and questions whether species other than cattle were intended. This rejection was discussed in more detail at the interview and various alternatives were analyzed. On further consideration, it is believed that the term suggested by the Examiner in the office action (i.e., cattle) is appropriate and clear, and accordingly, the claims have been amended to replace "bovine species" with --cattle--.

The Office Action also alleged that the declarations of Professors Janne and First may not be fully persuasive in the absence of a side-by-side comparison of the claimed *in vitro* methods vs. *in vivo* methods discussed in the cited references. This issue was discussed in more detail at the interview, and applicants pointed out that it would be impractical to provide data showing a side-by-side comparison and that the prior art could be distinguished without requiring submission of such evidence. It is believed that agreement was essentially reached on this point, subject to applicants briefly restating and clarifying previous arguments, as appears below.

The present claims are directed to generation of transgenic cattle by an *in vitro* method that entails obtaining ova from cattle ovaries, maturing the ova *in vitro*, fertilizing the ova *in vitro* to form a zygote, microinjecting the zygote *in vitro*, maturing the microinjected zygotes to form a preimplantation embryo *in vitro*, and then transplanted the embryo into a recipient female cattle. Differences between the claimed methods and the classical *in vivo* method used for generation of transgenic mice include: (a) in the claimed methods, maturation of oocytes prior to fertilization occurs *in vitro*, whereas in the classical method, maturation is induced in a live animal by superovulation; (b) fertilization is performed *in vitro* in the claimed methods and in a live animal in the classical method, (c) zygotes are matured into preimplantation embryos *in vitro* in the claimed methods, whereas in the *in vivo* method, zygotes are

transplanted into pseudopregnant animals for further maturation; and (d) the claimed methods can be performed without any surgical steps on live animals, whereas in the classical method surgery is required to extract fertilized oocytes and then to transplant microinjected zygotes into the pseudopregnant animals.

The claimed methods were nonobvious for at least the following reasons:

1. At the priority date of the invention (December 1989), there was no confirmed report in a peer-reviewed journal of a transgenic bovine having been produced by any method. In particular, attempts to extend the *in vivo* approach successfully used for generating transgenic mice to generation of transgenic cattle (Biery, Lostkutoff and Bondioli, all of record) had not resulted in viable transgenic cattle. See First declaration at paragraph 6(b).

2. Although an *in vitro* procedure for culturing cattle embryos had been discussed in the First patent, this patent did not describe the adaptation of this procedure to generation of transgenic cattle. See First declaration at paragraph 5.

3. There were a number of problems and uncertainties in attempting to combine the unsuccessful *in vivo* transgenesis procedure discussed by Biery, Lostkutoff and Bondioli with First's *in vitro* method. For example, it was unpredictable whether traditional microinjection procedures for *in-vivo* eggs could have been successfully applied without modification to *in-vitro* matured oocytes. It was also unpredictable whether the phasing of the cell of *in vitro* matured oocytes would have been different or impact on the visibility of the pronucleus and therefore on the injection protocol. Difficulty might also have arisen in determining the relative timing of microinjection and fertilization, which might not have been the same for *in vivo* and *in vitro* mature oocytes (First declaration at paragraph 6(b). It was also unpredictable whether the block on bovine development *in vitro* occurring at about the 8-cell stage could be overcome in the context of a transgenesis procedure (First declaration at paragraph 6(c). It was reasonable to expect that each of these difficulties and uncertainties would have required considerable

experimentation to overcome. Yet the ultimate end point for the efficacy of such experimentation would not have been apparent until several years later, so that practically, it was not possible to vary systematically most of the parameters (First declaration at paragraph 7).

4. Prior art requiring lengthy experimentation in varying multiple parameters without a reasonable expectation of ultimate success does not confer obviousness.

5. This analysis of the state of the art at the priority date of the invention is confirmed by several third party publications (referenced in the last response) acclaiming the invention.

In view of the above, it is respectfully submitted that the application is in condition for allowance. If the Examiner has any remaining concerns, a telephone conference is requested to discuss the same. Please call the undersigned at (415) 324 6318.

Respectfully submitted,

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